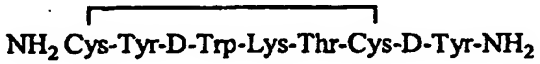


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| (54) Title: SOMATOSTATIN ANALOGS <div style="text-align: center;"> NH₂ Cys-Tyr-D-Trp-Lys-Thr-Cys-D-Tyr-NH₂ (1)</div> (57) Abstract <p>The present invention relates to somatostatin analogs which comprises a chemically substituted heptapeptide sequence having the cysteine groups in the 1 and 6 position being linked together to form a disulfide bridge in the monocyclic configuration. Chemical modifications are made at the free amino group of cysteine of the peptide (1). It has been demonstrated that the somatostatin analog peptide can be modified by the addition of isocyanates, isothiocyanates, acid chlorides, chloroformates and glycidyl ethers (epoxides) at the free amino group, at the terminal cysteine resulting in a measurable enhancement of the ability of the chemically modified compounds to bind somatostatin receptors.</p> | | |

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SOMATOSTATIN ANALOGS

BACKGROUND OF THE INVENTION

Somatostatin is a cyclic tetradecapeptide which inhibits release of several
5 pituitary and intestinal factors that regulate cell proliferation, cell motility, and/or
secretion including growth hormone, adrenocorticotropin hormone, prolactin, thyroid
stimulating hormone, insulin, glucagon, motilin, gastric inhibitory peptide, vasoactive
intestinal peptide, secretin, cholecystokinin, bombesin, gastrin releasing peptide, gastrin,
thyroid releasing hormone, pancreatic polypeptide, cytokines (e.g., interleukins,
10 interferons), growth factors (e.g., epidermal growth factor, nerve growth factor), and
vasoactive amines (e.g., serotonin). Several of these factors are implicated in regulation
of normal cell proliferation, as well as in tumor cell proliferation and metastasis.

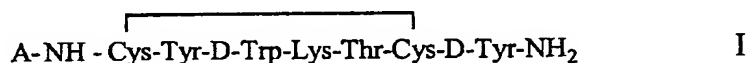
Native somatostatin has a very short half life in vivo. A large number of novel
analogues have been prepared in order to enhance the duration of effect, biological
15 activity and the selectivity of this hormone. A variety of somatostatin peptide analogs
have been produced by elimination of amino acids that are not absolutely required for
activity and substitution of the native L-amino acids with the corresponding D-amino
acid isomers. Thus, some of these analogs are long acting, more potent receptor agonists
than native somatostatin, due in part to the resistance of D-amino acids to enzyme
20 degradation. For example, the synthetic somatostatin analog octreotide acetate, which
has the amino acid sequence D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr(ol) is more potent

than native somatostatin in inhibition of growth factor release. Bauer et al. U.S. Patent No. 4,395,403.

SUMMARY OF THE INVENTION

5 The present invention provides novel chemically modified somatostatin analogs, structural derivatives of native somatostatin which bind a somatostatin receptor. Analogs include both antagonists and agonists of somatostatin activity.

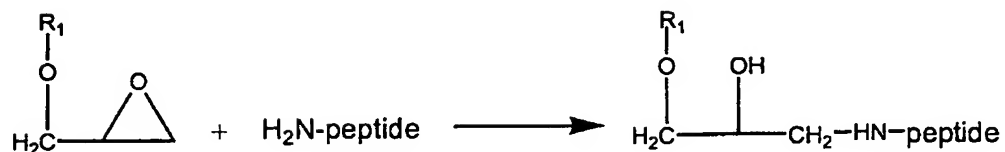
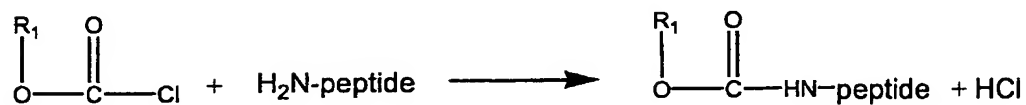
 More particularly, the present invention provides a somatostatin analog which comprises a chemically substituted heptapeptide sequence having the cysteine groups in
10 the 1 and 6 position being linked together to form an disulfide bridge in the monocyclic configuration. Chemical modifications are made at the free amino group of cysteine of the peptide below



15

 It has been demonstrated that the somatostatin analog peptide can be modified by the addition of isocyanates, isothiocyanates, acid chlorides, chloroformates and glycidyl ethers (epoxides) at the free amino group, at the terminal cysteine resulting in a measurable enhancement of the ability of the chemically modified compounds to bind
20 somatostatin receptors.

 The following synthetic reaction schemes are used to generate the chemically modified peptides.



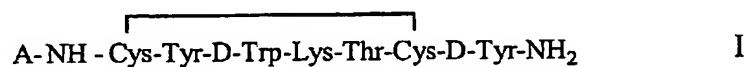
5 BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a graph which illustrates that peptide 3502 suppresses secretion of growth hormone.

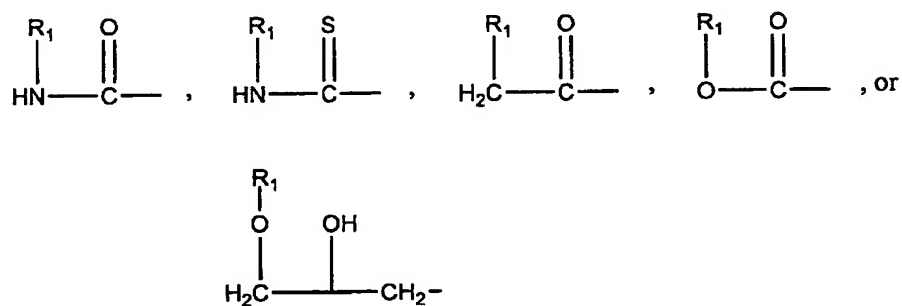
Figure 2 is a graph which shows that orally administered peptide 3502 prevents normal pulsatile secretion of growth hormone.

DETAILED DESCRIPTION OF THE INVENTION

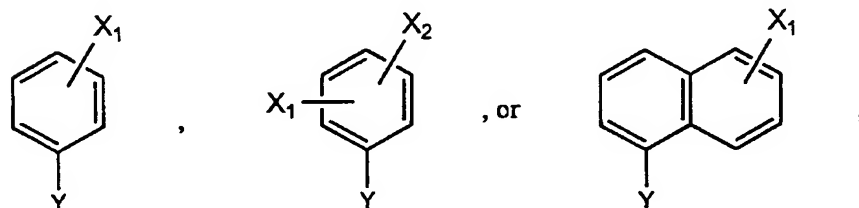
5 The compounds of this invention are cyclic heptapeptide analogs of somatostatin
having the general structure of Formula I



wherein A is

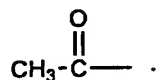


and R1 is C1-C4 alkyl, adamantyl,



Y is a bond, C1-C4 alkenyl, C=O, or SO₂; and

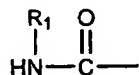
X_1 and X_2 are independently, flourine, chlorine, bromine, iodine, C1-C4 alkyl,
 NO_2 or



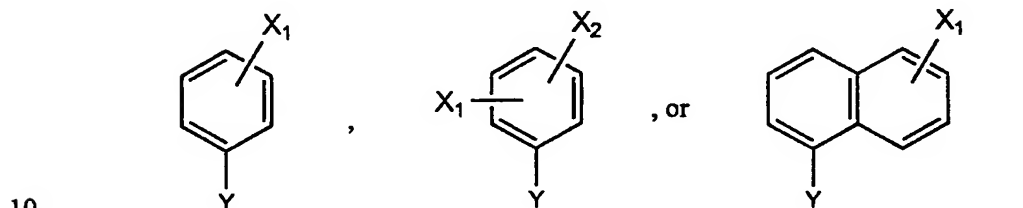
The chemical modifications of Formula I are generated at the free amino group of
 5 the terminal cysteine moiety of the heptapeptide sequence.

Preferred compounds of this invention include compounds of Formula I:

wherein A is

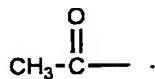


R_1 is C1-C4 alkyl, adamantyl,

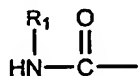


Y is a bond, C1-C4 alkenyl, $C=O$, or SO_2 ; and

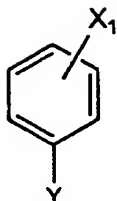
X_1 and X_2 are independently, flourine, chlorine, bromine, iodine, C1-C4 alkyl,
 NO_2 or



15 Another preferred compound of this invention of Formula I was the following
 structure wherein A is



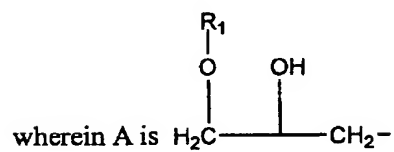
R₁ is



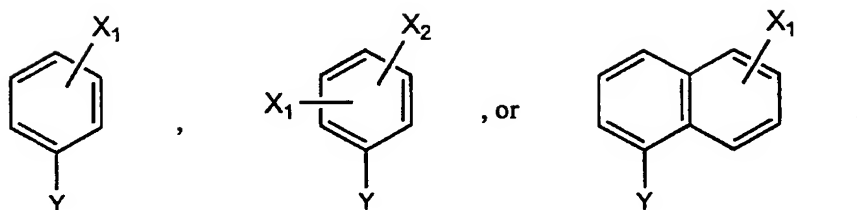
Y is CH₂ and

X₁ is hydrogen.

5 Other preferred somatostatin analogs are compounds of Formula I



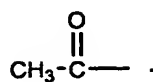
R₁ is C1-C4 alkyl, adamantyl,



10 Y is a bond, C1-C4 alkenyl, C=O, or SO₂ ; and

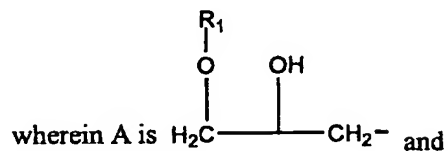
X₁ and X₂ are independently, fluorine, chlorine, bromine, iodine, C1-C4 alkyl,

NO₂ or

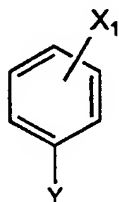


Another preferred somatostatin analog of this invention is a compound of

Formula I



5 R_1 is



X_1 is hydrogen, and

Y is a bond.

The invention features compounds, compositions and methods for the treatment
 10 of diseases in mammals associated with increased production or secretion of any factor
 or factors which can be regulated by somatostatin, including but not limited to growth
 hormone, insulin, glucagon and pancreatic exocrine secretion.

The compounds can be administered in the dosages used for somatostatin or,
 because of their greater potency, in smaller dosages. The compounds of the invention
 15 can be used for the treatment of cancer, particularly growth hormone- or growth factor-
 dependent cancer (e.g., bone, cartilage, pancreas, prostate, or breast), acromegaly and
 related hypersecretory endocrine states, or of bleeding ulcers and in those suffering from

pancreatitis or diarrhea. The compounds can also be used in the management of diabetes and to protect the liver of patients suffering from cirrhosis or hepatitis. The compounds can also be used to treat Alzheimer's disease and as gastric cytoprotective compounds for ulcer therapy. The compounds will also be useful in treating diabetes-related

5 retinopathy, nephropathy and vascular disease. The anti-cancer activity of the compounds may be related to their ability to antagonize the actions of cancer-related growth factors such as epidermal growth factor, insulin-like growth factor (IGF-1), or vasoactive endothelial growth factor (VEGF).

The analogs can be made available in the form of pharmaceutically acceptable

10 salts or complexes. Examples of therapeutically acceptable acids for the formulation of salts of the somatostatin analogs are inorganic acids, such as hydrochloric acid, sulfuric acid, phosphoric acid, and the organic lactic, maleic, citric, succinic, benzoic, salicylic, toluensulfonic acids. Complexes are compounds of Formula I formed by the addition of organic salts or hydroxides such as Ca and Zn salts or the addition polymeric organic

15 materials, such as tannic acid or carboxymethyl cellulose.

In other preferred embodiments, a therapeutically effective amount of the somatostatin analog or pharmaceutically acceptable salt or complex thereof are combined with a pharmaceutically acceptable carrier substance (e.g., magnesium carbonate, lactose, a phospholipid or mannitol) to form a pharmaceutical composition. Examples of

20 methods of administration of the therapeutic reagent of the pharmaceutical composition thereof include a pill, tablet, capsule or liquid for oral administration. The pharmaceutical composition can also be administered as an ointment, gel, cream or lotion

for application to the skin, or as a solution capable of being administered intravenously, parenterally, subcutaneously, transmucosally, intranasally or intraperitoneally in an appropriate buffer if necessary. The solid forms of this therapeutic composition can be coated with a substance capable of protecting the modified peptide from digestion by gastric acid in the stomach for a period of time sufficient to allow the composition to pass undisintegrated into the small intestine. The therapeutic composition can be administered via a sustained release formulation or a dermal patch. The descriptions are provided as examples and are not meant to limit the possibilities of therapeutic compositions or methods of administration of the somatostatin analogs.

10 Examples

The cyclic heptapeptide $\text{NH}_2 \text{Cys-Tyr-D-Trp-Lys-Thr-Cys-D-Tyr-NH}_2$ was purchased from Polypeptide Laboratories. The reagents used to modify the heptapeptide are widely available from commercial sources.

In accordance with the present invention, numerous modified peptides have been synthesized according to the synthetic schemes outlined below.

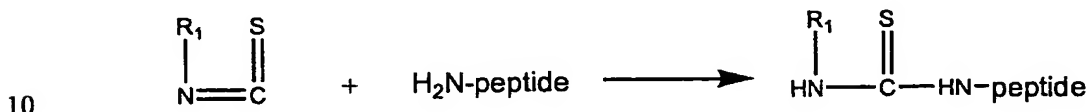
1) Reactions of isocyanate with the heptapeptide of Formula I:



The heptapeptide (as a trifluoroacetic acid salt) containing Boc-Lys was suspended in anhydrous acetonitrile to yield a 1 mM concentration. Fifty to 100 μl of

this mixture was placed in a microcentrifuge tube. One equivalent of triethylamine (100 mM in acetonitrile) was added with mixing, followed by the addition of 1 equivalent of the isocyanate (100 mM in acetonitrile). The reactions with the isocyanates were incubated at room temperature for 60 to 120 min. Ten μ l of water was added. The solvent was removed by evaporation under vacuum. The chemically modified peptides were then purified by reverse phase HPLC.

2) Reactions with isothiocyanates:



The heptapeptide (as a trifluoroacetic acid salt) containing Boc-Lys was suspended in anhydrous acetonitrile to yield a 1 mM concentration. Fifty to 100 μ l of this mixture was placed in a microcentrifuge tube. One equivalent of triethylamine (100 mM in a acetonitrile) was added with mixing, followed by the addition of one equivalent of isocyanate (100 mM in acetonitrile). The reactions with the isothiocyanates were incubated at room temperature for 18 hrs. The solvent was removed by evaporation under vacuum. The chemically modified peptides were then purified by reverse phase HPLC.

3) Reactions with acid chlorides:



The heptapeptide (as a trifluoroacetic acid salt) containing Boc-Lys was suspended in anhydrous acetonitrile to yield a 1 mM concentration. Fifty to 100 μl of this mixture was placed in a microcentrifuge tube. Two equivalents of triethylamine (100 mM in acetonitrile) was added with mixing, followed by the addition of 1 equivalent of an acid chloride (100 mM in acetonitrile). The reactions with the acid chlorides were incubated at room temperature for 60-120 min. The solvent was removed by evaporation under vacuum. The chemically modified peptides were then purified by reverse phase HPLC.

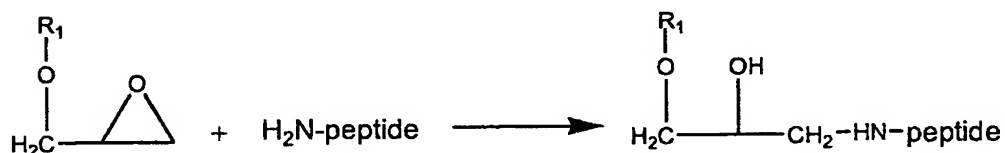
4) Reactions with chloroformates:



The heptapeptide (as a trifluoroacetic acid salt) containing Boc-Lys was suspended in anhydrous acetonitrile to yield a 1 mM concentration. Fifty to 100 μl of this mixture was placed in a microcentrifuge tube. Two equivalents of triethylamine (100 mM in acetonitrile) was added with mixing, followed by the addition of 1 equivalent of a chloroformate (100 mM in acetonitrile). The reactions with the

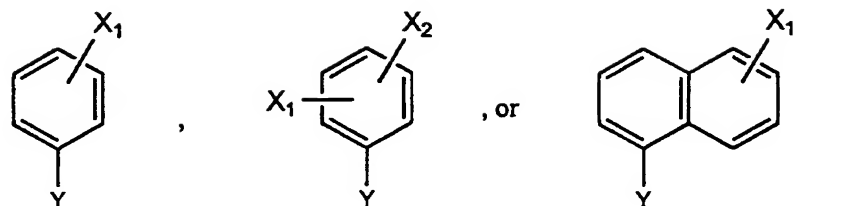
chloroformates were incubated at room temperature for 60-120 min. The solvent was removed by evaporation under vacuum. The chemically modified peptides were then purified by reverse phase HPLC.

5 5) Reactions with glycidyl ethers (epoxides):



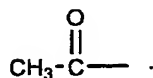
The heptapeptide (as a trifluoroacetic acid salt) containing Boc-Lys was suspended in anhydrous methanol to yield a 1 mM concentration. Fifty to 100 μl of this mixture was placed in a microcentrifuge tube. One equivalent of triethylamine (100 mM in acetonitrile), followed by the addition of 1 equivalent of a glycidyl ether (100 mM in methanol). The reaction was incubated at 65° C for 6-8 hrs. The solvent was removed by evaporation under vacuum. The chemically modified desired peptides were purified by reverse phase HPLC.

In the resulting modified peptides, R₁ is C1-C4 alkyl, adamantyl,



Y is a bond, C1-C4 alkenyl, C=O, or SO₂ ; and

- 5 X₁ and X₂ are independently, fluorine, chlorine, bromine, iodine, C1-C4 alkyl, NO₂ or



- HPLC purification of chemically modified amino acids. The dried reaction product was resuspended in 15μl of 100% of trifluoroacetic acid or 30 μl of 50% trifluoroacetic acid over a period of 5-10 minutes. The volume was brought to 100μl with 50% acetonitrile and the mixture was injected onto a C18 reverse phase HPLC column. The desired product was eluted from the column in a linear gradient of 0.1% trifluoroacetic acid in water and 0.1% trifluoroacetic acid in acetonitrile (B) progressing from 5% B at initial conditions to 60% B over 40 minutes. The elution position of the desired product was monitored by UV absorbance and the desired peak was collected by

hand in polypropylene tubes as it eluted from the UV detector. The eluent was dried under vacuum then used for binding and bioassays.

A variety of tissues and cancers express somatostatin receptors. Five human somatostatin receptors (hsst1, hsst2, hsst3, hsst4, hsst5) have been identified and cloned.

- 5 (Patel, Y.C., Life Sciences, Vol. 57, No. 13, pp. 1249-1265, 1995). Expression of these five receptor subtypes varies with tissue types. Somatostatin receptor subtype 2 is expressed on a wide variety of tumor types.

Receptor Binding Assays

- 10 Cell culture. CHO-K1 cells were grown as monolayers in Dulbecco's Modified Eagle's medium (DMEM, Mediatech, Washington DC) supplemented with 10% fetal calf serum, non-essential amino acids, 2mM glutamine, 1mM pyruvate and 500 mg/mL gentamycin in 5% CO₂ at 37°C.
- 15 Expression of hsst in CHO-K1 cells. For binding studies, CHO-K1 cell lines stably expressing hsst were created and propagated. The predicted coding region of each hsst was generated by PCR from human genomic DNA and oligonucleotides corresponding to the coding region 5' and 3' ends as primers. The DNA fragment generated by PCR contained a Hind III restriction site at the 5' end and a Not I restriction site at the 3' end. The fragment
- 20 was digested with these two restriction enzymes and directionally subcloned into the Hind III/Not I sites of the mammalian expression vector pCDNA1. The identity of each insert was verified by DNA sequencing. The construct was co-transfected with pSV2neo into a CHO-K1 cell line using the calcium phosphate protocol. Stable transfectants were selected using 400 mg/mL G418 and maintained in supplemented DMEM. After an initial ligand
- 25 binding screen, one stable clone for each sst was chosen for all subsequent experiments.

Preparation of Plasma Membranes. CHO/sst cells grown on 100mm tissue culture dishes were washed with ice cold PBS then scraped into 5ml of 50mM HEPES, pH 7.4-5mM $MgCl_2$ - 200 KIU/mL aprotinin - 2mg/mL PMSF and 2 mg/mL bacitracin (homogenization buffer). After a 15 min. incubation at 4°C, the cells were homogenized on ice using a
5 Brinkman Polytron (setting 5, 15 sec) then re-homogenized with a hand held homogenizer (6 strokes). After centrifugation at 500 x g for 5 min. at 4°C, the supernatant was centrifuged again at 12,000 x g for 25 minutes at 4°C. The final pellet was resuspended in homogenization buffer. Protein content was measured using the bicinchoninic acid protein assay using BSA as a standard.

10

Preparation of 96-well plates. Costar 96-well strip plates (cat. no. 9102) were coated with poly-l-lysine by incubating each well in 50 μ L 100mg/mL poly-l-lysine for 1hr at 22°C. Excess liquid was removed and the wells were air dried. Membranes (10mg/well) were added in 20mM Hepes pH 7.6 followed by incubation overnight at 4°C to evaporate all
15 liquid. Non-specific binding sites were blocked by incubating each well in 50 μ L homogenization buffer supplemented with 1% BSA (incubation buffer) for 30 min at RT. Binding assays were performed after removal of excess liquid.

[¹²⁵I-Tyr¹¹] SS14 binding. For receptor binding studies, membranes were incubated at

20

22°C with 0.03nM [¹²⁵I-Tyr¹¹] SS14 (obtained from Amersham) with or without test compounds each at concentrations ranging from 10⁻¹⁰ M to 10⁻⁶ M in 50 μ L incubation buffer. After a 1 hour incubation at 22°C, excess liquid was removed by gently tapping plates onto absorbent filter paper. Membranes were washed twice with 100 μ L ice cold incubation buffer and radioactivity in each well was determined. Specific binding was
25 defined as the difference between the amount of [¹²⁵I-Tyr¹¹] SS14 bound in the absence and presence of 1 μ M unlabeled SS14. Ki was determined using software programs Ligand or Prism.

The purified peptides were tested for binding to one or more of the five human somatostatin receptor subtypes. Table 1 lists the results of receptor binding studies for a number of peptides assayed against the five human somatostatin receptor subtypes. The data of Table 1 indicates the chemical modifications change the binding affinity of the parent heptapeptide to the various receptor subtypes.

TABLE 1

| # | Core Peptide Seq. | Reacting Compound | Ki's Human Receptors (nM) | | | | |
|------|---|--|---------------------------|--------|------|------|------|
| | | | sst1 | sst2 | sst3 | sst4 | sst5 |
| | Cys-Tyr-D-Trp-Lys-Thr-Cys-D-Tyr-NH ₂ | none | | 48.00 | | | |
| | Cys-Tyr-D-Trp-Lys-Thr-Cys-D-Tyr-NH ₂ | 1-Adamantyl isocyanate | | 2.65 | | | |
| 3502 | Cys-Tyr-D-Trp-Lys-Thr-Cys-D-Tyr-NH ₂ | Benzyl isocyanate | | 0.65 | 31.4 | 1566 | 3.5 |
| | Cys-Tyr-D-Trp-Lys-Thr-Cys-D-Tyr-NH ₂ | 4-Chlorophenyl isocyanate | 2000 | 0.90 | 31.5 | 1411 | 6.32 |
| | Cys-Tyr-D-Trp-Lys-Thr-Cys-D-Tyr-NH ₂ | 4-Methoxyphenyl isocyanate | 188 | 0.59 | 8.87 | 203 | 2.26 |
| | Cys-Tyr-D-Trp-Lys-Thr-Cys-D-Tyr-NH ₂ | (R)-(+)-alpha-Methylbenzyl isocyanate | 1940 | 0.41 | 21.5 | 1104 | 7.22 |
| | Cys-Tyr-D-Trp-Lys-Thr-Cys-D-Tyr-NH ₂ | (S)-(-)-alpha-Methylbenzyl isocyanate | 522 | 0.81 | 21 | 361 | 4.25 |
| | Cys-Tyr-D-Trp-Lys-Thr-Cys-D-Tyr-NH ₂ | (R)-(-)-1-(1-Naphthyl)ethyl isocyanate | | | | | |
| | Cys-Tyr-D-Trp-Lys-Thr-Cys-D-Tyr-NH ₂ | (S)-(+)-1-(1-Naphthyl)ethyl isocyanate | | 147.00 | | | |
| | Cys-Tyr-D-Trp-Lys-Thr-Cys-D-Tyr-NH ₂ | 4-Nitrophenyl isocyanate | 604 | 0.84 | 23.4 | 959 | 4.19 |
| | Cys-Tyr-D-Trp-Lys-Thr-Cys-D-Tyr-NH ₂ | Phenyl isocyanate | 778 | 0.44 | 12.1 | 769 | 3.88 |
| | Cys-Tyr-D-Trp-Lys-Thr-Cys-D-Tyr-NH ₂ | glycidyl 2-methylphenyl ether | | | | | |
| | Cys-Tyr-D-Trp-Lys-Thr-Cys-D-Tyr-NH ₂ | none | 1000 | 0.33 | 2 | 1000 | 8 |

| | | | | | | | |
|------|---|------------------------------------|------|------|------|------|------|
| | Cys-Tyr-D-Trp-Lys-Thr-Cys-D-Tyr-NH ₂ | 1,2 epoxy-3-phenoxypropene | 148 | | | | |
| | Cys-Tyr-D-Trp-Lys-Thr-Cys-D-Tyr-NH ₂ | [2,3 Epoxypropyl] benzene | 36 | | | | |
| | Cys-Tyr-D-Trp-Lys-Thr-Cys-D-Tyr-NH ₂ | 4-Chlorophenyl glycidyl ether | 7.7 | | | | |
| | Cys-Tyr-D-Trp-Lys-Thr-Cys-D-Tyr-NH ₂ | 4-tert-Butyl phenyl glycidyl ether | 12 | | | | |
| | Cys-Tyr-D-Trp-Lys-Thr-Cys-D-Tyr-NH ₂ | glycidyl 4-methoxyphenyl ether | | | | | |
| | Cys-Tyr-D-Trp-Lys-Thr-Cys-D-Tyr-NH ₂ | Benzenesulfonyl isocyanate | 1.9 | | | | |
| | Cys-Tyr-D-Trp-Lys-Thr-Cys-D-Tyr-NH ₂ | Benzoyl isocyanate | 3.4 | | | | |
| | Cys-Tyr-D-Trp-Lys-Thr-Cys-D-Tyr-NH ₂ | Benzoyl isothiocyanate | 2.9 | | | | |
| | Cys-Tyr-D-Trp-Lys-Thr-Cys-D-Tyr-NH ₂ | Benzyl isothiocyanate | 216 | | | | |
| | Cys-Tyr-D-Trp-Lys-Thr-Cys-D-Tyr-NH ₂ | 4-Chlorobenzenesulfonyl isocyanate | 3.7 | | | | |
| | Cys-Tyr-D-Trp-Lys-Thr-Cys-D-Tyr-NH ₂ | p-Toluenesulfonyl isocyanate | 2.1 | | | | |
| | Cys-Tyr-D-Trp-Lys-Thr-Cys-D-Tyr-NH ₂ | 4-Fluorophenyl isocyanate | 1093 | 1.34 | 38.6 | 1664 | 10.1 |
| | Cys-Tyr-D-Trp-Lys-Thr-Cys-D-Tyr-NH ₂ | Phenethyl Isothiocyanate | 103 | | | | |
| | Cys-Tyr-D-Trp-Lys-Thr-Cys-D-Tyr-NH ₂ | p-Tolyl isocyanate | 823 | 0.62 | 20.4 | 1120 | 6.36 |
| | Cys-Tyr-D-Trp-Lys-Thr-Cys-D-Tyr-NH ₂ | Trifluoro-p-tolyl isocyanate | 3.3 | | | | |
| | Cys-Tyr-D-Trp-Lys-Thr-Cys-D-Tyr-NH ₂ | 4-Bromophenyl isocyanate | 1206 | 1.05 | 31.5 | 1204 | 8.64 |
| | Cys-Tyr-D-Trp-Lys-Thr-Cys-D-Tyr-NH ₂ | 2-Phenylphenyl isocyanate | 454 | 0.92 | 50 | 799 | 10.4 |
| | Cys-Tyr-D-Trp-Lys-Thr-Cys-D-Tyr-NH ₂ | Phenethyl isocyanate | | | | | |
| | Cys-Tyr-D-Trp-Lys-Thr-Cys-D-Tyr-NH ₂ | 2,6-Dimethylphenyl isocyanate | | | | | |
| | Cys-Tyr-D-Trp-Lys-Thr-Cys-D-Tyr-NH ₂ | 4-Phenoxyphenyl isocyanate | 1.9 | | | | |
| 3533 | Cys-Tyr-D-Trp-Lys-Thr-Cys-D-Tyr-NH ₂ | 4-Acetylphenyl isocyanate | 1522 | 0.21 | 29.9 | 1147 | 5.65 |
| | Cys-Tyr-D-Trp-Lys-Thr-Cys-D-Tyr-NH ₂ | 4-n-Butylphenyl isocyanate | 2.6 | | | | |

| | | | | | | |
|---|------------------------------------|------|------|------|-----|------|
| Cys-Tyr-D-Trp-Lys-Thr-Cys-D-Tyr-NH ₂ | 4-Chloro-2-methylphenyl isocyanate | 1 | | | | |
| Cys-Tyr-D-Trp-Lys-Thr-Cys-D-Tyr-NH ₂ | 2,4-Dichlorobenzyl isocyanate | 1.2 | 14.4 | 591 | 1 | |
| Cys-Tyr-D-Trp-Lys-Thr-Cys-D-Tyr-NH ₂ | 2,3-Dimethylphenyl isocyanate | 0.69 | 8.4 | 353 | 3.9 | |
| Cys-Tyr-D-Trp-Lys-Thr-Cys-D-Tyr-NH ₂ | 2,4-Dimethylphenyl isocyanate | 0.7 | 6.9 | 251 | 2.8 | |
| Cys-Tyr-D-Trp-Lys-Thr-Cys-D-Tyr-NH ₂ | 2,5-Dimethylphenyl isocyanate | 0.62 | 8.1 | 284 | 2 | |
| Cys-Tyr-D-Trp-Lys-Thr-Cys-D-Tyr-NH ₂ | 3,4-Dimethylphenyl isocyanate | 0.77 | 11.7 | 105 | 3.9 | |
| Cys-Tyr-D-Trp-Lys-Thr-Cys-D-Tyr-NH ₂ | 3,5-Dimethylphenyl isocyanate | 1334 | 0.43 | 10.7 | 561 | 3.03 |
| Cys-Tyr-D-Trp-Lys-Thr-Cys-D-Tyr-NH ₂ | 4-Ethylphenyl isocyanate | 0.81 | 12.1 | 274 | 3.1 | |
| Cys-Tyr-D-Trp-Lys-Thr-Cys-D-Tyr-NH ₂ | p-Toluoyl chloride | 1.35 | 17.6 | 340 | 1.7 | |
| Cys-Tyr-D-Trp-Lys-Thr-Cys-D-Tyr-NH ₂ | Cinnamoyl chloride | 1.01 | 19.3 | 432 | 4.5 | |
| Cys-Tyr-D-Trp-Lys-Thr-Cys-D-Tyr-NH ₂ | Phenyl chloroformate | 2.58 | | | | |
| Cys-Tyr-D-Trp-Lys-Thr-Cys-D-Tyr-NH ₂ | Benzyl chloroformate | | | | | |
| Cys-Tyr-D-Trp-Lys-Val-Cys-D-Tyr-NH ₂ | none | 18 | | | | |
| Cys-Tyr-D-Trp-Lys-Val-Cys-D-Tyr-NH ₂ | benzyl isocyanate | 1.8 | | | | |
| Cys-Tyr-D-Trp-Lys-Val-Cys-D-Tyr-NH ₂ | Phenyl isocyanate | 4.1 | | | | |
| Cys-Tyr-D-Trp-Lys-Val-Cys-D-Tyr-NH ₂ | 4-Acetylphenyl isocyanate | 1.9 | | | | |

Bioactivity Assays

The in vivo biological activity of modified peptides was determined by evaluating their inhibitory potency on pituitary growth hormone (GH) release in sodium pentobarbital-anesthetized rats. The pentobarbital treated rat is a well characterized and frequently used model for studying GH secretory dynamics (see K. Chihara, A. Armura and AV Schally 1979 Endocrinology 104 1434).

Dose-Response Studies: Adult male Sprague-Dawley rats weighing 250-300g with jugular vein cannulas were obtained from Zivic-Miller Labs, Zelienople, PA. On the day of assay, the rats were anesthetized with sodium pentobarbital (60mg/kg of body weight, administered i.p.). Thirty minutes later, the animals were injected iv. with saline or test compound at doses ranging from 0.1 to 30 $\mu\text{g/kg}$. Blood samples (250 μL) were drawn from the jugular vein cannula 10 min prior to test compound injection (baseline) and 5, 15, 30, 45 and 60 minutes after injection. The plasma was separated and assayed for GH by RIA using material supplied by the National Hormone and Pituitary Program, and for glucagon and glucose using commercially available reagents. To prevent hemodynamic disturbances, the red blood cells were resuspended in normal saline and returned to the animal. Figure 1 illustrates the dose response of peptide 3502 at three dosage levels 5 $\mu\text{g/kg}$, 2.5 $\mu\text{g/kg}$, and 1 $\mu\text{g/kg}$. At each dosage, the peptide was shown to be effective in suppressing production of growth hormone.

Time-Course Assay: Groups of cannulated rats were treated with sodium pentobarbital as in the dose-response assay. Thirty minutes later animals were injected via the jugular cannula with saline or test compound at the minimum dose giving maximal GH inhibition. Sodium pentobarbital at half the initial dose was given at 60- to 90-minute intervals to maintain anesthesia. Blood (250 μL) was collected from the jugular vein at approximately 15, 30, 60, 120, 180, and 240 min. after the injection of test compound and treated as described above. Data from the time-course assay are shown in Table 2. The results of this experiment demonstrates that compounds 3502 and 3533 block the ability of arginine to stimulate glucagon secretion, mimicking a normal function of somatostatin. The alpha cells which secrete glucagon are known to express the SST₂ receptor; thus this activity of the peptides is consistent with their selectivity for the SST₂ receptor.

The data also show that the peptides do not cause hypoglycemia, either alone or in combination with arginine.

| Time | Treatment | Assay: Glucagon (pg/ml) | | GH (ng/ml) | | Glucose (mg/dl) | |
|---------|-----------------------|-------------------------|------|------------|-------|-----------------|------|
| | | mean | sem | mean | sem | mean | sem |
| -35 min | pentobarbital 70mg/kg | | | | | | |
| -15 min | | | | | | | |
| -10 min | saline | | | | | | |
| -5 min | | | | | | | |
| 0 min | L-arg. .400mg/kg | | | | | | |
| 5min | | 47.1 | 8.6 | 255.4 | 175.4 | 153.1 | 8.1 |
| 10 min | | | | | | | |
| 15 min | | 40.6 | 4.5 | 64.5 | 32.3 | 140.4 | 8.8 |
| 30min | | 102.1 | 7.2 | 43.9 | 18.4 | 160.3 | 10.9 |
| 45 min | | 82.8 | 7.7 | 28.4 | 10.9 | 162.0 | 14.4 |
| 60 min | | 63.7 | 0.9 | 26.5 | 10.8 | 136.7 | 13.8 |
| 75 min | | 49.7 | 6.1 | 46.0 | 14.4 | 103.1 | 11.9 |
| | | 45.1 | 3.6 | 57.4 | 13.8 | 91.9 | 5.1 |
| | | 41.2 | 4.4 | 59.1 | 4.2 | 103.9 | 3.0 |
| | | 42.7 | 10.8 | 54.3 | 8.2 | 107.2 | 2.6 |

| Time | Treatment: | Assay: Glucagon (pg/ml) | | GH (ng/ml) | | Glucose (mg/dl) | |
|---------|-----------------------|-------------------------|------|------------|-------|-----------------|-----|
| | | mean | sem | mean | sem | mean | sem |
| -35 min | pentobarbital 70mg/kg | | | | | | |
| -15 min | | | | | | | |
| -10 min | MS3502, 5ug/kg | | | | | | |
| -5 min | | | | | | | |
| 0 min | L-arg. .400mg/kg | | | | | | |
| 5min | | 50.2 | 2.0 | 283.7 | 208.8 | 152.5 | 1.9 |
| 10 min | | | | | | | |
| 15 min | | 43.1 | 8.3 | 72.1 | 31.2 | 143.7 | 3.2 |
| | | 76.2 | 8.8 | 24.3 | 8.1 | 162.4 | 4.0 |
| | | 62.8 | 12.6 | 12.7 | 3.5 | 150.3 | 8.3 |
| | | 38.9 | 18.0 | 9.7 | 2.4 | 128.4 | 7.7 |

| | | | | | | |
|--------|------|------|------|------|------|-----|
| 30min | 29.9 | 11.8 | 6.4 | 0.8 | 89.9 | 6.5 |
| 45 min | 34.1 | 10.5 | 13.4 | 5.9 | 75.9 | 8.0 |
| 60 min | 34.6 | 10.0 | 22.5 | 11.3 | 88.0 | 7.0 |
| 75 min | 37.5 | 4.6 | 27.0 | 10.7 | 98.7 | 3.2 |

Treatment:

| | | | | | | |
|---------|-----------------------|------|------|-------|-------|-------|
| Time | | | | | | |
| -35 min | | | | | | |
| -15 min | | | | | | |
| -10 min | | | | | | |
| -5 min | | | | | | |
| 0 min | | | | | | |
| 5min | | | | | | |
| 10 min | | | | | | |
| 15 min | | | | | | |
| 30min | | | | | | |
| 45 min | | | | | | |
| 60 min | | | | | | |
| 75 min | | | | | | |
| | pentobarbital 70mg/kg | 50.1 | 8.4 | 284.3 | 230.8 | 157.7 |
| | MS3533, 5ug/kg | 40.4 | 7.5 | 79.0 | 46.9 | 150.0 |
| | L-arg. ,400mg/kg | 73.2 | 11.6 | 34.8 | 23.5 | 162.2 |
| | | 65.2 | 10.2 | 28.1 | 18.0 | 156.5 |
| | | 46.1 | 4.0 | 20.3 | 10.5 | 138.6 |
| | | 38.0 | 2.4 | 12.2 | 3.6 | 100.9 |
| | | 37.5 | 6.1 | 12.5 | 3.8 | 88.9 |
| | | 39.2 | 8.2 | 14.5 | 7.5 | 88.8 |
| | | 46.3 | 7.9 | 15.1 | 6.8 | 103.4 |

7.1

2.3

8.7

1.2

6.0

4.5

1.7

8.4

14.6

Treatment:

| | | | | | | |
|---------|-----------------------|------|-----|------|------|-------|
| Time | | | | | | |
| -35 min | | | | | | |
| -15 min | | | | | | |
| -10 min | | | | | | |
| -5 min | | | | | | |
| 0 min | | | | | | |
| | pentobarbital 70mg/kg | 40.8 | 5.7 | 89.9 | 45.7 | 153.4 |
| | MS3502, 1ug/kg | 35.1 | 3.2 | 41.9 | 16.4 | 145.7 |
| | L-arg. ,400mg/kg | | | | | |

7.7

0.9

| Time | 95.6 | 11.6 | 26.1 | 9.9 | 161.6 | 5.9 |
|--------|------|------|------|------|-------|------|
| 5min | 95.6 | 11.6 | 26.1 | 9.9 | 161.6 | 5.9 |
| 10 min | 72.2 | 4.2 | 21.2 | 5.7 | 162.4 | 14.0 |
| 15 min | 50.9 | 3.7 | 18.8 | 5.7 | 126.8 | 8.8 |
| 30min | 43.9 | 6.3 | 39.5 | 18.4 | 91.9 | 5.4 |
| 45 min | 43.5 | 6.0 | 43.4 | 5.9 | 77.7 | 3.0 |
| 60 min | 36.2 | 4.2 | 65.5 | 14.9 | 90.1 | 4.8 |
| 75 min | 36.6 | 7.8 | 60.4 | 17.7 | 102.3 | 0.1 |

| Time | Treatment: | | | | | |
|---------|-----------------------|-------|------|-------|------|-------|
| -35 min | pentobarbital 70mg/kg | | | | | |
| -15 min | | 49.5 | 4.9 | 137.0 | 73.1 | 157.2 |
| -10 min | MS3533, 1ug/kg | | | | | |
| -5 min | | 41.7 | 6.1 | 61.8 | 21.0 | 144.6 |
| 0 min | L-arg. ,400mg/kg | | | | | |
| 5min | | 111.0 | 17.5 | 32.2 | 6.4 | 159.2 |
| 10 min | | 77.3 | 10.2 | 23.5 | 5.1 | 151.5 |
| 15 min | | 59.8 | 3.2 | 19.3 | 1.0 | 128.3 |
| 30min | | 48.8 | 4.4 | 40.6 | 13.7 | 93.5 |
| 45 min | | 47.9 | 5.7 | 53.1 | 21.8 | 89.6 |
| 60 min | | 39.6 | 8.4 | 45.3 | 14.3 | 99.2 |
| 75 min | | 33.8 | 17.0 | 24.7 | 12.4 | 76.0 |
| | | | | | | 38.2 |

| Time | Treatment: | 57.0 | 3.76 | 46.9 | 10.2 | 170.1 | 12.3 |
|---------|-----------------------|------|------|------|------|-------|------|
| -35 min | pentobarbital 70mg/kg | | | | | | |
| -15 min | | | | | | | |
| -10 min | MS3502, 5ug/kg | | | | | | |

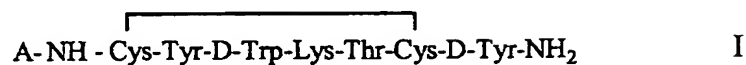
Oral activity: Adult male Sprague-Dawley rats weighing 250-300g with jugular vein and gastric cannulas were obtained from Zivic-Miller Labs, Zelienople, PA. On the evening prior to assay, rats were given 5 gram of food to eat with free access to water. On the day of assay, the rats were anesthetized with sodium pentobarbital (60mg/kg of body weight, administered i.p.). Thirty minutes later, the animals were injected through the gastric cannula with saline or test compound at doses ranging from 0.1 to 30 $\mu\text{g/kg}$ in a total volume of 200 μL . Sodium pentobarbital at half the initial dose was given at 60- to 90-minute intervals to maintain anesthesia. Blood (250 μL) was collected from the jugular vein at approximately 15, 30, 60, 120, 180, and 240 min. after the injection of test compound and treated as described above. In Figure 2 the graph representing oral saline illustrates the cyclic increase and decrease of growth hormone levels during normal secretion. The graph representing peptide 3502 shows that the peptide, orally administered, prevents the normal secretion of growth hormone.

15

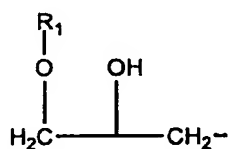
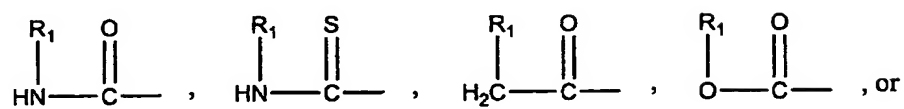
20

WHAT IS CLAIMED IS:

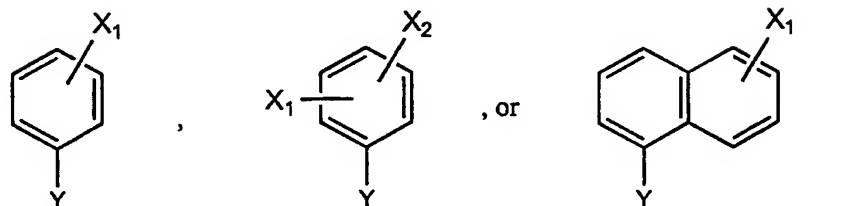
1. A modified heptapeptide of Formula I



- 5 wherein A is

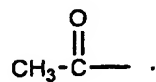


and R₁ is C1-C4 alkyl, adamantyl,



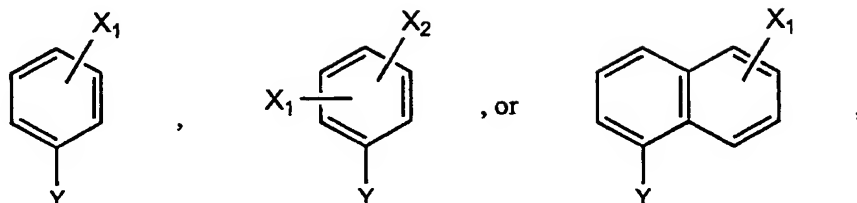
Y is a bond, C1-C4 alkenyl, C=O, or SO₂; and

- 10 X₁ and X₂ are independently hydrogen, fluorine, chlorine, bromine, iodine, C1-C4 alkyl, NO₂ or



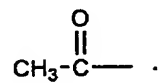
2. The modified heptapeptide of Claim 1 wherein A is $\text{HN}-\overset{\text{R}_1}{\overset{|}{\text{C}}}-\overset{\text{O}}{\overset{||}{\text{C}}}-$

and R_1 is C1-C4 alkyl, adamantyl,



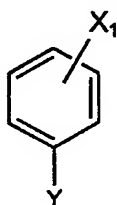
5 Y is a bond, C1-C4 alkenyl, C=O, or SO₂; and

X_1 and X_2 are independently, fluorine, chlorine, bromine, iodine, C1-C4 alkyl, NO₂ or



3. The modified heptapeptide of Claim 2, wherein

10 R_1 is

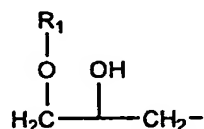


Y is CH₂ and

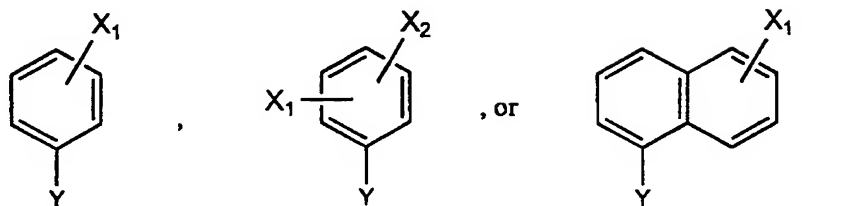
X_1 is hydrogen.

15

4. The modified heptapeptide of Claim 1, wherein A is



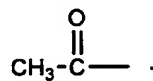
R₁ is C1-C4 alkyl, adamantyl,



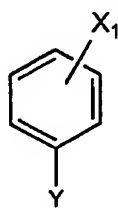
5 Y is a bond, C1-C4 alkenyl, C=O, SO₂; and

X₁ and X₂ are independently, fluorine, chlorine, bromine, iodine, C1-C4 alkyl,

NO₂ or



10 5. The modified heptapeptide of Claim 4 wherein



R₁ is

Y is a bond and X₁ is hydrogen.

6. A pharmaceutical composition comprising a compound of Formula I, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

7. A pharmaceutical composition comprising a modified heptapeptide of
5 Claim 3, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

8. A pharmaceutical composition comprising a modified heptapeptide of
Claim 5, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable
10 carrier.

9. A method for inhibiting the release of growth hormone, insulin, and glucagon in a mammal comprising administering to the mammal a modified heptapeptide of Formula I.

Acute GH Suppression by Somatostatin Agonists

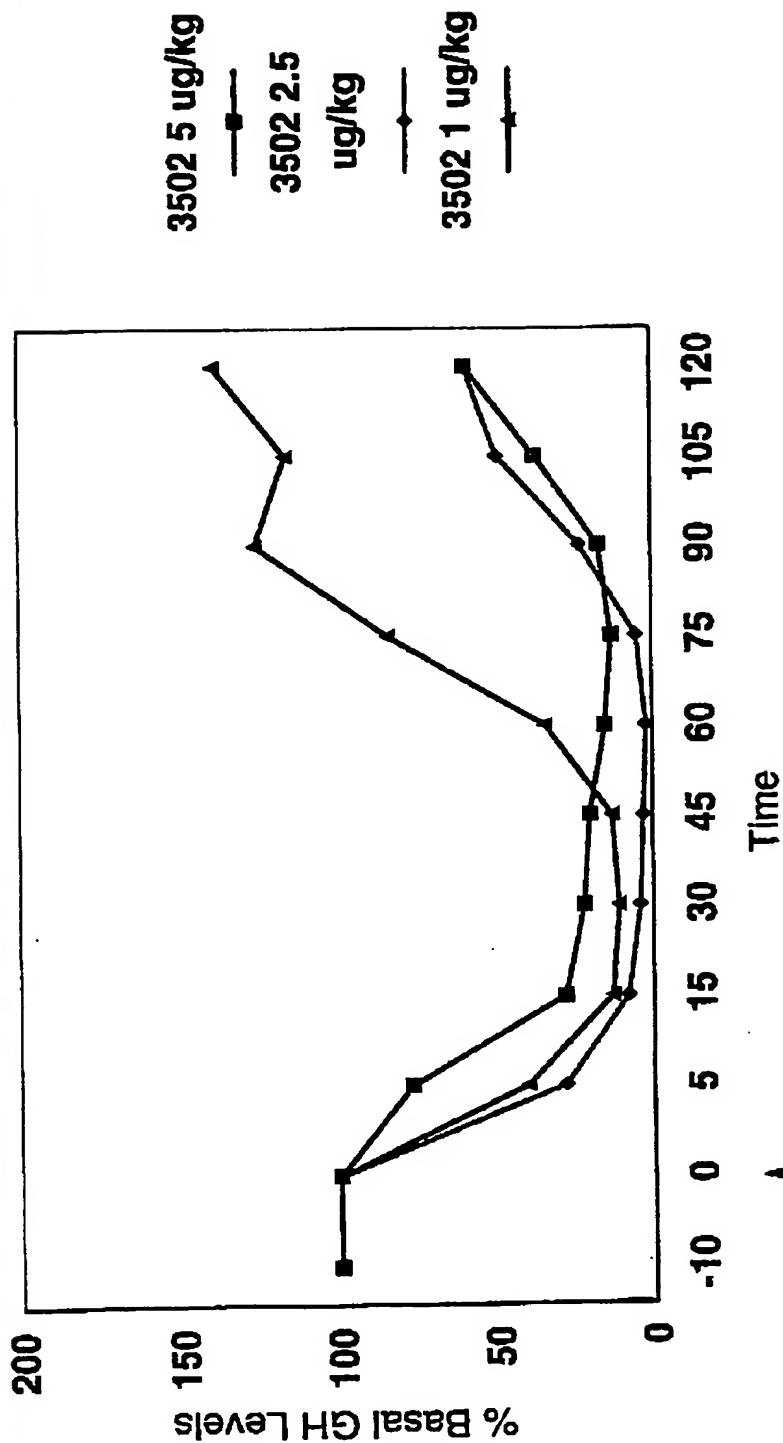


FIGURE 1

2/2

GH Pulsatility & Oral Somatostatin Agonist Treatment

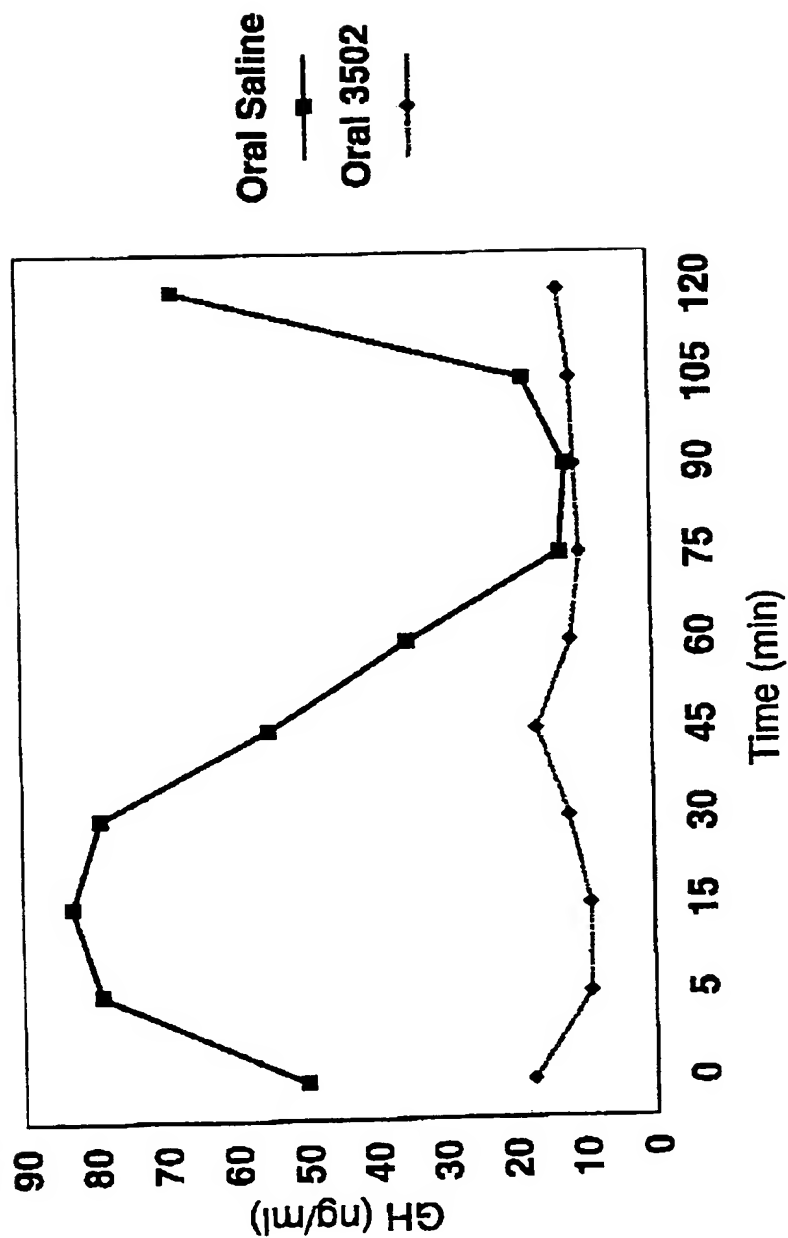


FIGURE 2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/19090

| A. CLASSIFICATION OF SUBJECT MATTER IPC(7) : A61K 38/00, 38/31, 38/12; C07K 5/00, 7/00 US CL : 514/9, 11, 16; 530/300, 311, 317, 329 According to International Patent Classification (IPC) or to both national classification and IPC | | | | | | | | | | | | | | |
|---|--|---|--|---|--|--|--|--|---|---|--|--|--|--|
| B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) U.S. : 514/9, 11, 16; 530/300, 311, 317, 329 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) CAS ONLINE, USPATFULL, BIOSIS, MEDLINE. | | | | | | | | | | | | | | |
| C. DOCUMENTS CONSIDERED TO BE RELEVANT | | | | | | | | | | | | | | |
| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. | | | | | | | | | | | | |
| A | US 4,145,337 A (DAIRMAN et al) 20 March 1979, see entire document. | 1-9 | | | | | | | | | | | | |
| A | US 5,770,687 A (HORNIK et al) 23 June 1998, see entire document. | 1-9 | | | | | | | | | | | | |
| A,P | US 5,846,934 A (BASS et al) 08 December 1998, see entire document. | 1-9 | | | | | | | | | | | | |
| <input type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex. | | | | | | | | | | | | | | |
| <table border="0"><tr><td>* Special categories of cited documents:</td><td>*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</td></tr><tr><td>*A* document defining the general state of the art which is not considered to be of particular relevance</td><td>*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</td></tr><tr><td>*B* earlier document published on or after the international filing date</td><td>*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</td></tr><tr><td>*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</td><td>*A* document member of the same patent family</td></tr><tr><td>*O* document referring to an oral disclosure, use, exhibition or other means</td><td></td></tr><tr><td>*P* document published prior to the international filing date but later than the priority date claimed</td><td></td></tr></table> | | | * Special categories of cited documents: | *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention | *A* document defining the general state of the art which is not considered to be of particular relevance | *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone | *B* earlier document published on or after the international filing date | *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art | *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) | *A* document member of the same patent family | *O* document referring to an oral disclosure, use, exhibition or other means | | *P* document published prior to the international filing date but later than the priority date claimed | |
| * Special categories of cited documents: | *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention | | | | | | | | | | | | | |
| *A* document defining the general state of the art which is not considered to be of particular relevance | *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone | | | | | | | | | | | | | |
| *B* earlier document published on or after the international filing date | *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art | | | | | | | | | | | | | |
| *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) | *A* document member of the same patent family | | | | | | | | | | | | | |
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